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14. ABSTRACT The goal of this project is to develop a novel means to inhibit prostate cancer development and progression. The development of Siah1/2 inhibitors to the ubiquitin ligase Siah1/2 has been advanced by the ability to develop a Siah1/2 inhibitory peptide that effectively inhibits Siah1/2 activity, which was found to effectively attenuate the growth of prostate cancer tumors <i>in vivo</i> when transplanted subcutaneously or orthotopically into the prostate site. The assessment of the Siah1/2 inhibitory peptide in genetic models of mouse as in human PDX tumors is ongoing by the Partnering PIs Drs. Martin Gleave and Neil Bhowmick at the two respective sites. Parallel development of small molecule inhibitors to Siah2 is on going and will complement the work performed with the inhibitory peptide. This is a first-in-class inhibitor of the ubiquitin ligase that can be administered intravenously and that has a notable effect on prostate cancer growth <i>in vivo</i> .					
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1. INTRODUCTION

Being the most diagnosed malignancy in men and the second leading death of cancer-related diseases, prostate cancer (PCa) remains a significant clinical challenge (Wyatt and Gleave, 2015). PCa initially responds to the first line androgen deprivation therapy (ADT) or androgen receptor (AR) pathway inhibition (ARPI) but eventually develops into lethal castration resistance prostate cancer (CRPC, Lorient et al., 2012). The most recognized AR-negative CRPC variant is neuroendocrine PCa (NEPC), which is characterized by the expression of neuroendocrine markers such as chromogranin A (CHGA), synaptophysin (SYP) and neuron-specific enolase (NSE). NEPC is highly aggressive and poorly diagnosed, and the mechanisms underlining trans-differentiation of NEPC remain elusive. Therapeutic targeting of NEPC is challenging due in part to their aggressiveness and similarity to neuronal cells. There is an urgent unmet need for mechanistic understanding and novel therapy candidates for this lethal disease variant (Toren and Gleave, 2013).

Among those mechanisms being tested, the Siah2 protein has shown significant support on the progression of CRPC (Qi et al., 2013) and NEPC (Qi et al., 2010). Playing the role of an ubiquitin E3 ligase, Siah2 selectively triggers degradation of a subset pool of inactive AR therefore promoting expression of a sub-pool of AR target genes (Qi et al., 2013). Siah2 also facilitates the ubiquitination and degradation of prolyl hydroxylase 3 (PHD3), hence allows stabilization of the HIF1 α protein (Nakayama et al., 2004) and modulates the expression of HIF1 α -associated genes (Qi et al., 2010; Nakayama et al., 2004). Furthermore, Siah2 regulates the tight junction integrity and cell polarity under hypoxia conditions by modulating availability of protein ASPP2 (Kim et al., 2014). Siah2 is markedly increased in CRPC and Siah2 inhibition promotes prostate cancer regression upon castration (Qi et al., 2013). Therefore, Siah2 has become a promising therapeutic target for CRPC and NEPC (Qi et al., 2013; Qi et al., 2010).

The first year of this project was devoted to advance the generation of Siah inhibitors, and we approached this in two parallel ways. We succeeded in developing promising inhibitors that can be used for the evaluation of Siah inhibition *in vivo*. To this end, we are using four *in vivo* models to evaluate the impact of these inhibitors on PCa development and progression, with a focus on CRPC and NEPC.

Dr. Ronai performs the initial evaluation in the human adenocarcinoma of the prostate, CW22RV1, which is able to form NEPC and metastasizes to lymph nodes and lungs. The second model is assessed by Dr. Gleave, who has developed the Shionogi mouse model, a mouse androgen-dependent mammary carcinoma that, like human prostate cancer, regresses after castration and later recurs as an androgen-independent tumor. In this model, androgen-dependent tumors in intact mice undergo complete regression following androgen ablation, but rapidly growing androgen-independent tumors recur after 1 month in a highly reproducible manner (Bruchovsky et al., 1990). Therefore, this model is particularly useful to evaluate the efficacy of agents targeting progression to androgen independence (Miayake et al., 2000). The third *in vivo* model, which is also assessed in Dr. Gleave's laboratory, is the patient derived xenograft (PDX) model, which is highly clinical relevant (Lin et al., 2014). The PDX LTL352 model was developed from a patient's metastatic poorly differentiated NEPC. When grafted under the renal capsules of NOD-SCID mice, LTL352 xenografts show androgen-independent tumor growth and invade into adjacent host kidney parenchyma and metastases to distant organs. It expresses typical neuroendocrine markers (e.g., CHGA and SYP with absence of expression of AR). Another PDX model LTL331 was derived from hormonal naive prostatic adenocarcinoma tissue and retained key properties of the original tumor, including histopathological, genomic and transcriptomic characteristics. Castration of mice carrying LTL331 tumors leads to a decrease in tumor volume and plasma PSA levels. However, tumors recur after 6–8 months. This recurrent tumor line, designated LTL331R (which will be used in this study) is highly proliferative and showed androgen-independent growth. It was entirely AR and PSA negative, uniformly expressed a range of neuroendocrine markers, including SYP, CHGA, CHGB and CD56. Importantly, recent clinical follow-up information showed that the patient, from whom the LTL331 line had been derived, developed NEPC after long-term androgen ablation therapy. The fourth tumor model is evaluated by Dr. Bhowmick, who utilizes the Beige-SCID mice hosting tissue recombination orthotopic grafts having 22Rv1 prostatic epithelia with human carcinoma associated fibroblasts (CAF).

2. KEYWORDS

Prostate cancer (PCa); castration resistant PCa; neuroendocrine PCa; Siah1/2; ubiquitin ligases; androgen receptor; HIF1a, CRPC, NEPC, patient derived xenograft (PDX); Shionogi model

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1: Develop and assess the activity of Siah2 inhibitors

Major Task 1: Further assessment of SBI-0640601.

Major Task 2: Develop additional derivatives that exhibit superior biophysical properties.

Major Task 3: Assess SBI-0640601 analogs in benchmark pharmacology, pharmacokinetic, and toxicology studies in cultured cells

Major Task 4: Select best performing SBI-0640601 analogs (3-5) for studies in mice.

Major Task 5: Select best SBI-0640601 analog for in vivo assessment in PCa mouse models.

Milestone #1: Identify at least one small molecule that is equal if not more potent than SBI-0640601 for use in Specific Aims 2 and 3.

Milestone #1 has been completed on schedule.

Specific Aim 2: Test available Siah2 inhibitors in relevant PCa cultures

Major Task 1: Assess Siah2 inhibitors in relevant NEPC cultures.

Major Task 2: Assess Siah2 inhibitors in relevant ACP-NE cultures.

Major Task 3: Assess Siah2 inhibitors in relevant CRPC cultures.

Milestone #2: Establish efficacy of Siah2 inhibitors in each of the tumor models and identify whether inhibition of Siah2 alone or in combination with currently used drugs has equal or preferable effect on one of the major PCa types assessed.

Specific Aim 3: Test Siah2 inhibitors in PCa models in vivo

Major Task 1: Determine the effect of Siah2 inhibitors (alone and in combination with existing therapies) in xenograft models of castrate resistant, neuroendocrine, and metastatic PCa.

Major Task 2: Test the efficacy of Siah antagonists in the prevention of castration therapy resistance development in novel transgenic and xenograft model systems.

Major Task 3: Determine the effect of Siah2 inhibitors on prostatic and bone metastatic stromal microenvironment on CRPC development.

Major Task 4: Evaluate the efficacy of Siah2 inhibitors on PDX of PCa.

Major Task 5: Determine the ability of Siah2 inhibitor to inhibit CRPC conversion to NE phenotypes when combined with existing therapies.

Milestone #3: We will establish which of the different PCa tumors best responds to Siah2 inhibition, alone or in combination with currently available therapies, monitoring development, progression (metastasis) as well as the conversion of CRPC to NE.

What was accomplished under these goals?

Specific Aim 1 – Develop and assess the activity of Siah2 inhibitors

Major Task 1: Further assessment of SBI-0640601; months 1–3 (Dr. Ronai and Dr. Pinkerton)

This task has been fully completed, as we finalized the characterization of SBI-601, substantiating its effects on prostate cancer cells in culture. These assays included colony forming efficiency, soft agar growth, and assessment of Siah-signature biomarkers, including HIF1 α and select AR-target genes. This analysis also raised initial concerns regarding the possibility that there may not be a direct effect of SBI-601 on Siah1/2, leading us to develop alternative inhibitors for these ubiquitin ligases.

Major Task 2: Develop initial additional derivatives that exhibit superior biophysical properties; months 1–6 (Dr. Ronai and Dr. Pinkerton)

We proceeded as planned and synthesized six additional derivatives of SBI-601 (SBI-0646847, SBI-0646849, SBI-0646850, SBI-0646-851, SBI-0646852, SBI-0646853). Given that these are natural compound-based structures, the development of derivatives is not trivial and required substantial work, which was performed at the Sanford Burnham Prebys (SBP) CPCCG. At least one of these derivatives (SBI-0646852) appeared to exhibit superior biophysical properties compared with the parent compound, SBI-601. This task was accomplished within 4 months.

The parent and newly developed analogs were subject to a series of rigorous assessments per their effect on Siah1/2 in both culture and in vitro, using purified proteins. Unfortunately, all our efforts to establish direct interaction or an effect of the parent or analog compounds on Siah2 failed. This led us to conclude that these compounds elicit their biological effect via a different mechanism, which although mimics Siah1/2 own signature, does not engages Siah1/2 directly. This conclusion, coupled with limited effect in in vivo assessments (see below) prompted us to initiate new campaigns for the development of Siah1/2 inhibitors.

Thus, over the subsequent 8 months, we devoted significant efforts towards the two following tasks, which have resulted in promising positive outcomes.

First, we started a new campaign to identify small molecule Siah inhibitors, albeit, by relying on newly developed technology that became available in the CPCCG. This approach relies on the ability of a small molecule to alter the conformation and biophysical properties of the protein it binds to, a change that can be captured using a thermal protein shift assay (PTS). The change in the conformation results in the change of the melting temperature of the protein, which can then be captured in high throughput format. One of the requirements of this assay is the use of a relatively large amount of purified protein. We thus set to produce over 100 mg of the purified Siah1 protein, which was accomplished within 4 months. We were then able to calibrate the conditions for the PTS assay for the Siah1 protein. This allowed us to proceed and perform a HTP screen for Siah1 small molecule inhibitors, in a scale of 32,000 compounds. The assay has been carried out and led to the identification of 17 hits that significantly affected the melting temperature of Siah1 protein. Those 17 hits were then assessed in cell culture for their effect on Siah1/2 surrogate markers—HIF1, AR select genes, as well as the effectiveness of these compounds on the growth and toxicity towards prostate cancer cells. Of the 17 compounds we identified, three (SBI-0087259, SBI-0089054, and SBI-0098687) exhibited a more potent effect and are currently undergoing further assessment in biochemical assays.

Second, we set to advance a Siah inhibitory peptide that we recently developed in parallel, as summarized in our 2013 publication in Chemical Biology. At that stage the inhibitory peptide was 35-45 amino acids long, although the backbone of the actual inhibitory peptide was about 11 amino acids. This peptide was limited to culture work due to its length and the difficulty to produce it in large amounts hindered possible assessment in vivo. We thus initiated a campaign to improve on the Siah1/2 inhibitory peptide, which was performed over an 8-month period through a series of six iterative steps. Therein we deleted the penetratine sequence with modified amino acids allowing membrane penetration, by reducing the polyproline linker, without impacting the effect and efficacy of

its ability to inhibit Siah, and assuring that the peptide will have a long half life in the cells. These changes were successfully incorporated and at present we have generated a short peptide that effectively inhibits Siah1/2 in culture as well as in vivo (130B3). The selectivity and specificity of this peptide to Siah1/2 is secured through the design to force covalent binding of the peptide to Siah1/2 protein. Such covalent association was confirmed in Maldi-TOFF based analysis and more so, in structural studies where the crystal structure of Siah1/2 together with its inhibitory peptide were mapped.

Major Task 3: Assess SBI-0640601 analogs in benchmark pharmacology, pharmacokinetic, and toxicology studies in cultured cells; months 1–12 (Dr. Ronai and Dr. Pinkerton)

Due to the changes performed in Major Task 2, the assessment of the newly identified Siah1/2 inhibitory small molecules will be performed over months 12–15 months.

The assessment of the Siah1/2 inhibitory peptide for benchmark pharmacology pharmacokinetic and toxicology is currently being performed. We expect to complete these over months 12–15 of the funding period.

Major Task 4: Select best performing SBI-0640601 analogs (3–5) for studies in mice; months 8–14 (Dr. Ronai and Dr. Pinkerton)

We subjected the SBI-601 and SBI-852 for analysis in mice, as proposed. We found that the effect of these compounds on in vivo growth of the PC tumor CDW22RV1 is limited. This finding together with the lack of our ability to establish direct effect on Siah ubiquitin ligase activity, prompted the alternative routes we undertook, as detailed above.

Of note, one of the interesting observations made in our in vivo assessment is the ability of SBI-852 to attenuate the neuroendocrine phenotype seen in RV1 cells in vivo. Further, a similar observation was made by our colleague Dr. Bhowmick at Cedars-Sinai (see report below).

Major Task 5: Select best SBI-0640601 analog for in vivo assessment in PCa mouse models; months 6–14 (Dr. Ronai, Dr. Liddington, Dr. Bhowmick and Dr. Gleave)

We performed an initial assessment of the Siah1/2 inhibitory peptide in both culture and in vivo, using sc injected RV1 cells and using orthotopically injected cells. We administered the peptide over a 3-week period, using daily iv injections (5 days injection with 2 days off before the next series of 5 daily injection proceed). In both, the orthotopic and sc tumors, we found that the peptide (administered along the same protocol for both modes) effectively inhibited the growth of the prostate tumor in vivo. Notably, a substantially greater effect was observed in the orthotopically injected tumor cells, which failed to produce a tumor, compared with the control. In the case of the sc injected tumor, the degree of inhibition was about 50% over a 3-week period. In all cases, the peptide was administered iv at a dose of 10 mg/kg. In vivo assessment of the peptide effect on additional PC cultures will be performed over the first 2–3 months of the second year of funding.

To produce sufficient material for in vivo studies, we synthesized and purified 1 gram of the Siah1/2 inhibitory peptide, which has now been distributed to both Dr. Bhowmick and Dr. Gleave.

Milestone 1: Identify at least one small molecule that is equal if not more potent than SBI-0640601/SBI-852 for use in Specific Aims 2 and 3.

This milestone has been reached as we identified and confirmed the specificity and effectiveness of a Siah1/2 inhibitory peptide, which was successfully modified to enable its effectiveness in vivo.

Specific Aim 2 – Test available Siah2 inhibitors in relevant PCa cultures

Major Task 1: Assess Siah2 inhibitors in relevant NE-PC cultures; months 6–24 (Dr. Ronai, Dr. Bhowmick and Dr. Gleave)

*We have begun the assessment of Siah1/2 inhibitory peptide using five different prostate tumor cultures representing also the NE-PC. The peptide exhibited effective inhibition of the NE-PC lines, with RV1 serving as one example (see appendix, **Figures 1 and 2**).*

Major Task 2: Assess Siah2 inhibitors in relevant ACP-NE cultures; months 6–24 (Dr. Ronai, Dr. Bhowmick and Dr. Gleave)

We have begun the assessment of Siah1/2 inhibitory peptide using five different prostate tumor cultures representing also the APC-NE. The peptide exhibited effective inhibition of these PC lines.

Major Task 3: Assess Siah2 inhibitors in relevant CRPC cultures, months 6–24 (Dr. Ronai, Dr. Bhowmick and Dr. Gleave)

*We have begun the assessment of Siah1/2 inhibitory peptide using five different prostate tumor cultures representing also the CRPC. The peptide exhibited effective inhibition of these PC lines, which were monitored by toxicity and growth on 3D (see appendix, **Figures 2 and 3**).*

Milestone 2: Establish efficacy of Siah2 inhibitors in each of the tumor models and identify whether inhibition of Siah2 alone or in combination with currently used drugs has equal or preferable effects on one of the major PCa types assessed.

This milestone is on target to be completed during the second year of funding.

Specific Aim 3 – Test Siah2 inhibitors in PCa models in vivo

Major Task 1: Determine the effect of Siah2 inhibitors (alone and in combination with existing therapies) in xenograft models of castrate resistant, neuroendocrine, and metastatic PCa; months 12–24 (Dr. Ronai, Dr. Bhowmick and Dr. Gleave)

*Initial results with Siah inhibitory peptide demonstrate its ability to attenuate growth of RV1 prostate cancer cells when administered sc (see appendix, **Figure 4**) or by orthotopic prostate injections (see appendix, **Figure 5**). We expect to complete this milestone over the second year of this funding period, as originally planned.*

We expect to complete this milestone over the second year of this funding period, as originally planned.

Major Task 2: Test the efficacy of Siah antagonists in the prevention of castration therapy resistance development in novel transgenic and xenograft model systems; months 12–24 (Dr. Bhowmick and Dr. Gleave)

Dr. Gleave's lab selected to use the Shionogi model given the marked increase in Siah2 expression in this tumor (90% fulfilled).

*Siah2 protein levels were examined in pre-existing tumor protein samples obtained from Shionogi tumors before castration (day 0), and post castration (day 3, 5, 7, 10, and 13). Western blotting suggests that Siah2 protein level was acutely induced by day 3 after castration, rapidly reached a peak level by day 7, and then calms down by day 10 to day 13 while still maintained a significantly high levels compared to non-castrated tumors (see appendix, **Figure 6A**). The NEPC markers SYP and NSE are also found induced after castration, but at a later stage (day 5 and day 7, respectively)*

compared to Siah2 (day 3). Another NEPC marker CHGA is not altered along the whole period examined (see appendix, **Figure 6A**). mRNA levels of Siah2 and NSE are also induced after castration, but displayed different induction time courses compared to the protein levels (see appendix, **Figure 6B**). Interestingly, HIF1 α mRNA levels is also induced post castration (see appendix, **Figure 6C**), and its protein levels is being examined in protein lysates. Two antibodies against mouse HIF1 α have been tested but both didn't give specific bands in Shionogi tumor samples. We will test other antibodies to screen the HIF1 α protein levels in the model. The repression of tumor volumes after castration is shown in **Figure 6C** (see appendix). Expression of Siah2 in PDX model also reveals notable increase in the expression levels (see appendix, **Figures 7 and 8**) as detailed below.

Dr. Gleave studies of a Siah2-inhibiting compound 852 for its affects on repression and recurrence of Shionogi tumor after castration (75% fulfilled).

Procedure:

1. 5×10^6 of TD-2 cells will be injected subcutaneously into DD/S mice and tumor growth will be screened biweekly in three dimensions using calipers.

2. When tumors reach 500 mm^3 (2 to 3 weeks after injection) mice will receive castration under anaesthesia and randomly enter group A (vehicle), group B (20 mg/kg of compound 852) and group C (40 mg/kg of 852). Compound 852 treatment will start from the day after castration (day 1). Tumor size will be continuously measured biweekly until the sacrifice date.
[852 compound: 20 and 40 mg/kg, i.p, three doses/week.]

3. Data analysis will be focused on the following aspects:

- Tumor volumes among the three groups.
- Siah2 protein levels by western blot and IHC
- HIF1 α protein levels by western blot and IHC
- NE markers (NSE, SYP and CHGA) by western blot and IHC
- AR target genes which Siah2 affects – including PMEPA1, NKX3.1, PSA

Current status: All treatments have been finished and tumors harvested. As shown in **Figure 9** (see appendix), we didn't observe significant differences among the vehicle and compound 852 treated group. Detailed analysis is undergoing to explore if the compound 852 inhibited functions of Siah2, by examining the protein levels of HIF1 α and NEPC markers NSE, CHGA and SYP.

Dr. Gleave's evaluation of Siah2-inhibiting peptide 130B3 in repression and recurrence of Shionogi tumor after castration (50% fulfilled).

Procedure:

1. 5×10^6 of TD-2 cells will be injected subcutaneously into DD/S mice and tumor growth will be screened biweekly in three dimensions using calipers.

2. When tumors reach 500 mm^3 (2 to 3 weeks after injection) mice will receive castration under anesthesia and randomly enter group A (vehicle), group B (10 mg/kg of 130B3). Peptide 130B3 treatment will start from the next day (day 1) and tumor size will be continuously measured biweekly until the sacrifice date.
[130B3: 10 mg/kg, iv, three doses/week]

3. Data analysis will be focused on the following aspects:

- Tumor volumes among the three groups.

- *Siah2* protein levels by western blot and IHC
- *HIF1α* protein levels by western blot and IHC
- NE markers (NSE, SYP and CHGA) by western blot and IHC
- AR target genes which *Siah2* affects – including *PMEPA1*, *NKX3.1*, *PSA*

Current status: The Shionogi tumor model has been set up, the treatment with 130B3 is undergoing, and tumor volumes are being closely monitored.

Dr. Gleave determined whether the *Siah2* level is induced in NEPC samples (100% fulfilled).

To investigate if *Siah2* level is enhanced in NEPC tumors, we carried out IHC staining against *Siah2* in a TMA set, which includes clinical patients' samples ("AB") and PDX xenograft tumors ("LTL"). As shown in **Figure 7A** and **7B** (see appendix), *Siah2* protein levels were induced in 6 out of 7 NEPC samples (in red), comparing to the adenocarcinoma specimens (in blue). IHC shows nuclear staining of *Siah2* protein in the tumors (see appendix, **Figure 7B**). However, *Siah2* mRNA levels are found reduced in PDX LTL331 model after castration (Cx) (see appendix, **Figure 8A**); and no significant difference on *Siah2* mRNA is detected between NEPC and adenocarcinoma in Mark Rubin's database (see appendix, **Figure 8B**). This suggests that *Siah2* is tightly regulated by multiple mechanisms at both transcription and post-transcriptional levels, and the expression data needs to be carefully interpreted.

Dr. Gleave's evaluation of 130B3 for its ability to retard growth of NEPC in LTL352 and LTL331 models (30% fulfilled).

Procedure:

1. Recovery of LTL331R and LTL352 (NEPC patient) xenograft from frozen stock. LTL331R or LTL352 tumor tissues will be recovered from liquid nitrogen stocks and maintained in male NOD-SCID mice under the renal capsules with supplement of testosterone. Recovery period varies from 12–15 weeks.
2. Generation of LTL331R or LTL352 xenografts in pre-castrated mice. Tissue pieces of recovered 331R or 352 will be grafted under the renal capsules of 30 pre-castrated male mice. Allow tumors to grow and adapt to their new microenvironment in mice for 6–8 weeks.
3. *Siah2* inhibitor 130B3 treatment. When tumors reach 500 mm³, mice will be randomly assigned into group A (vehicle) and group B (130B3, 10 mg/kg, iv, three doses/week). And tumor size will be continuously measured biweekly until the sacrifice date.
4. Data analysis will be focused on the following aspects:
 - Tumor volumes among the three groups
 - *Siah2* protein levels by western blot and IHC
 - *HIF1α* protein levels by western blot and IHC
 - NE markers (NSE, SYP and CHGA) by western blot and IHC
 - AR target genes which *Siah2* affects – including *PMEPA1*, *NKX3.1*, *PSA*

Current status: LTL352 model has been set up, the 130B3 treatment is undergoing, and tumor volumes are under close monitoring. LTL331 model is being recovered (step 1).

During the next reporting period, we will continue the work described above and aim at:

- Determine *HIF1α* protein levels in Shionogi tumors.
- Finish the data analysis on compound 852 treated Shionogi tumors.

- *Finish the treatment of 130B3 on Shionogi model and start the data analysis.*
- *Finish the 130B3 treatment on LTL352 model and start the data analysis; and try to set up the LTL331R model for 130B3 treatment.*

Major Task 3: Determine the effect of Siah2 inhibitors on prostatic and bone metastatic stromal microenvironment on CRPC development; months 12–24 (Dr. Bhowmick)

*Dr. Bhowmick's lab has tested two Siah-selective compounds in xenograft mouse models. They were both tested in Beige-SCID mice hosting tissue recombination orthotopic grafts having 22Rv1 prostatic epithelia with human carcinoma associated fibroblasts (CAF). In the first study (n = 16) mice were castrated 4 weeks following grafting to initiate a neuroendocrine response by the tumor. Subsequently, Siah inhibitory compound SBI-852 was administered at (20 mg/kg for two weeks. While no appreciable differences in tumor volume were found among the treatment groups, there was a dramatic change in the differentiation state of the tumors. We found that that chromogranin A expression that was reproducibly expressed in the vehicle treated mice was largely absent in the mice treated with SBI-852 (see appendix, **Figure 10**). Analogously, the observed down regulation of androgen receptor (AR) potentiated by castration, seemed to be restored in the tumors in mice treated with SBI-852. However, there were minimal differences in proliferation (Ki-67, phosphor-histone H3) or cell death (Tunel) markers in the treatment and control mice (data not shown). Dr Bhowmick next tested the efficacy of 130B3, a very different compound on a similar mouse model system. With demonstrated greater selectivity for Siah activity, we expected 130B3 to provide greater efficacy on gross tumor expansion. In the following study we grafted the 22Rv1/CAF tissue recombinants, allowed the tumors to expand for four weeks, then simultaneously administered enzalutamide (40 mg/kg, standard of care androgen receptor antagonist) with 130B3 (10 mg/kg) daily. The enzalutamide was given by oral gavage, while the 130B3 was given by tail vein. This resulted in a survival analysis study due to some toxicity observed with enzalutamide administration (n = 8). We found that the mice treated with a combination of 130B3 and enzalutamide had improved survival compared to those treated with enzalutamide alone (see appendix, **Figure 11**). It should be noted that since in men, enzalutamide associated toxicity not especially pronounced we would likely need to reduce the dose of enzalutamide in future experiments to determine if such a survival difference is clinically relevant. While gross tumor size among the treatment groups did not differ, the orthotopic tumors showed in the mice treated with 130B3 and enzalutamide had elevated necrosis, compared to those treated with enzalutamide alone (see appendix, **Figure 12**). Additionally, Dr. Bhowmick found like with the SBI-852 compound, that neuroendocrine differentiation was significantly down regulated with 130B3 administration. In future experiments there is a plan to extend the treatment course longer (with lower enzalutamide doses, e.g., 1 mg/kg and 10 mg/kg) to potentially find differences in gross tumor size and metastatic progression. The pronounced reduction in neuroendocrine differentiation of PCa tumors is significant finding that could suggest other potential combination treatment therapeutics like taxanes).*

We expect to complete this task over the second year of this funding period, as originally planned.

Major Task 4: Evaluate the efficacy of Siah2 inhibitors on PDX of PCa; months 12–24 (Dr. Bhowmick and Dr. Gleave)

We expect to complete this task over the second year of this funding period, as originally planned.

Major Task 5: Determine the ability of Siah2 inhibitor to inhibit CRPC conversion to NE phenotypes when combined with existing therapies; months 12–24 (Dr. Ronai and Dr. Gleave)

We expect to complete this task over the second year of this funding period, as originally planned.

Milestone 3: We will establish which of the different PCa tumors best responds to Siah2 inhibition, alone or in combination with currently available therapies, monitoring development, progression (metastasis) as well as the conversion of CRPC to NE.

We expect to complete this milestone over the second year of this funding period, as originally planned.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to the communities of interest?

The results of the studies performed during the first year of this funding period were discussed in professional meetings including the ubiquitin conference that was held in Croatia in September 2015. A discussion with the prestigious forum at UCSD, named Oher, provided an opportunity to discuss our approach and results with the greater community members (May 2015).

What do you plan to do during the next reporting period to accomplish the goals?

We have made changes during the first year of funding to assure we are able to achieve the goals of this proposal at the best possible way. We have paved the road for the success of the proposed program by implementing two parallel approaches, thereby securing our ability to complete these goals, as outlined above. We expect that our work in concert with our two collaborators will enable to successfully complete the originally planned experiments.

4. IMPACT

The work performed during the first year of this project can be considered as a paradigm shift in cancer research in general and prostate cancer in particular. To this day there is no specific inhibitor of a ubiquitin ligase which can be administered in vivo, as we have now accomplished by the work we performed with the Siah1/2 peptide inhibitors. We have further used a state of the art approach to screen for novel Siah1/2 small molecule inhibitors, which led to identification of several promising compounds we currently subject to rigorous assessment as part of the originally planned studies.

Our continued collaboration with Drs. Gleave and Bhowmick will be of particular importance during the second year of this proposal when they are evaluating the significance of inhibiting Siah1/2 for the most aggressive prostate cancer, CRPC and NE type. CRPC and NEPC remain major obstacles in treatment of advanced PCa. Our study will explore roles of Siah2 on CRPC development and on growth of established NEPC. The PDX and Shionogi models recruited in our study present highly clinical relevance and provide unique systems on evaluating the efficacy of agents targeting progress of advanced disease. Data obtained from our study using Siah2 inhibiting agents, compound 852 and peptide 130B3, will help to determine if targeting Siah2 may bring significant benefit to patients with CRPC and NEPC.

What was the impact on the development of the principal disciplines of the project?

The need to alter our original plan due to disappointing results forced the incorporation of two alternate approaches, each is unique and first in class on its own, which were successfully implemented within this short time allowing progress of the originally planned studies.

What was the impact to other disciplines?

Our work over the first year have secured our ability to perform our planned studies in the best possible way, using distinct novel approaches which offer a paradigm shift in development and therapeutic modalities for PC.

What was the impact on technology transfer?

We expect that the outcome of our work during the first year will offer novel intellectual properties and technologies that will be disseminated to the greater community.

What was the impact on society beyond science and technology?

The ability to develop a first in class reagents to inhibit PC

5. CHANGES/PROBLEMS

Changes in approach and reasons for change.

As outlined above, we had to alter the original plan to develop the SBI-601 and its analogs further given their poor performance in vivo and in light of the inability to confirm their direct impact on the Siah1/2 ligases. This has prompted our development of two alternate approaches that appear to be successful and secure the continued work, as originally planned, in defining a new class of inhibitors to the most aggressive forms of PC.

Actual or anticipated problems or delays and actions or plans to resolve them.

Nothing to report.

Changes that have a significant impact on expenditures.

The change in the approach described above has impacted the expenditures of this project, which was reflected in cost to synthesize sizeable amount of Siah1 protein, the cost of synthesizing number of Siah1/2 inhibitory peptides, the cost of synthesizing large amount of the select Siah1/2 inhibitory peptide (1 gram) and the cost of establishing and performing the thermal shift assay on 32,000 compounds.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

6. PRODUCTS

Publications, conference papers, and presentations

The results of the work performed over the first year has so far been described in a couple of ubiquitin and cancer meetings, including the EMBO ubiquitin conference in Croatia in September 2015 and a ubiquitin workshop in China in June this year. Patent applications are expected upon further refining the Siah1/2 inhibitory peptide and the small molecules identified in the course of the HTP screen we performed this year.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**What individuals have worked on the project?****Ronai Lab**

Name:	Ze'ev Ronai
Project Role:	Principal Investigator
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	PI
Funding Support:	N/A

Name:	Yongmei Feng
Project Role:	Staff Scientist
Researcher Identifier:	
Nearest person month worked:	6
Contributions to Project:	Performed assessment of inhibitors in culture and <i>in vivo</i> .
Funding Support:	N/A

Name:	Anthony Pinkerton
Project Role:	Medicinal Chemist, CPCCG
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	Synthesized novel Siah1/2 inhibitors
Funding Support:	N/A
Name:	Marzia Scortegagna
Project Role:	Staff Scientist
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	Performed analysis of select Siah inhibitors.
Funding Support:	N/A

Name:	Eugenio Santelli
Project Role:	Staff Scientist
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Produced Siah1/2 protein for HTP screen
Funding Support:	N/A

Name:	Marilyn Leonard
Project Role:	Lab Manager
Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	Managed animal colony and lab supplies/reagents.
Funding Support:	N/A

Gleave Lab

Name:	Fan Zhang
Project Role:	Research Associate
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Dr. Zhang is the project manager for this study. Dr. Zhang has been communicating with Ronai's lab, Gleave's lab and Wang's lab for the protocol preparation. Dr. Zhang has been manipulating the animal work together with the animal staff at Vancouver Prostate Centre, and Dr. Zhang is in charge of the data analysis and report preparation.
Funding Support:	N/A

Name:	Alexander Kretschmer
Project Role:	Post-doctoral Researcher
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Dr. Kretschmer has been taking the duty to monitor tumor growth and animal body weight and helps to harvest tissues at the end point of treatments. Dr. Kretschmer is also heavily involved with data analysis.
Funding Support:	N/A

Name:	Mary Bowen
Project Role:	Research Assistance on animal work
Researcher Identifier:	
Nearest person month worked:	2
Contributions to Project:	Ms. Bowen sets up the animal models and performs the drug treatments.
Funding Support:	N/A

Name:	Dong Lin
Project Role:	Research Associate in Dr. YZ Wang's lab

Researcher Identifier:	
Nearest person month worked:	1
Contributions to Project:	Dr. Lin has been cooperating to set up the PDX LTL352 and 331R tumor models for the study.
Funding Support:	N/A

Bhowmick Lab

Name:	Manisha Tripathi
Project Role:	Postdoctoral Fellow
Researcher Identifier:	
Nearest person month worked:	8.4
Contributions to Project:	Performed the surgeries and helped in the analysis of the tissues.
Funding Support:	N/A

Name:	Rajeev Mishra
Project Role:	Postdoctoral Fellow
Researcher Identifier:	
Nearest person month worked:	3
Contributions to Project:	Helped in the surgical procedures, treatment of the mice, and analysis.
Funding Support:	N/A

Name:	Neil Bhowmick
Project Role:	Principal Investigator
Researcher Identifier:	
Nearest person month worked:	0.5
Contributions to Project:	Design and analysis of the data. Share responsibility of overall running of the project and coordinating with Drs. Gleave and Ronai.
Funding Support:	N/A

As we have developed new peptides and established the ability to perform novel screen for Siah1/2 inhibitors we have engaged additional in-house collaborators that enabled us to secure our progress to date. This includes Dr. Christian Hassig (working with Dr. Anthony Pinkerton) to oversee the development of the HTP screen and its performance, as well as Drs. Surya De and Maurizio Pellecchia, who assisted in design and synthesis of the novel Siah1/2 inhibitory peptides.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Ze'ev Ronai, Initiating PI

Grants that have ended:

Melanoma Research Foundation (PI: Ronai, Z.) 10/01/13–09/30/15 0.0 calendar (0%)
Understanding and Targeting ER Stress Pathways in Melanoma
Goals: This proposal focuses on characterizing the Siah2-ER stress pathway in melanoma, thereby

contributing to our understanding of the mechanisms underlying melanoma resistance, which in turn will lead to the development of novel therapies.

Specific Aims: (1) Characterize the deregulated ER stress response in a subset of BRAF WT melanomas. (2) Establish the significance of the Siah2-ER stress regulatory axis in melanoma development and response to chemotherapy. (3) Determine the effect of inhibitors of the Siah2-ER stress axis on melanoma development and response to chemotherapy.

Scientific Officer: Shelby Moneer
Melanoma Research Foundation
1411 K Street, NW Suite 800
Washington, DC 20005
Phone: (202) 347-9675
E-mail: smoneer@melanoma.org

5 R01 CA111515 (PI: Ronai, Z.) 07/01/10–04/30/15 1.2 calendar (10.0%)
NIH/NCI

Novel Insights in the Regulation of HIF1 α Stability

Goals: The proposed studies will identify mechanisms underlying FoxA2 and Siah2 forms of prostate tumors. By characterizing the roles of FoxA2 and Siah2 these studies will provide insight into mechanisms underlying HIF activity and demonstrate the importance of these activities to prostate NE lesions/tumors that are known to be the more aggressive form of prostate cancer.

Specific Aims: (1) Determine the role of FoxA2, HIF-1 α and Siah in human prostate tumor development and progression. (2) Characterize mechanisms underlying FoxA2 cooperation with HIF-1 α . (3) Determine the role of select HIF-1 α /FoxA2 regulated genes in prostate tumor development and progression. (4) Identify key domains required for FoxA2 association and cooperation with HIF-1 α to determine whether inhibiting FoxA2/HIF-1 α cooperation by use of select peptides blocks formation of NE phenotype and prostate tumors in mouse xenograft models using human CWR22Rv1 and LNCaP cells.

Grants Officer: Angela Urdaneta
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Phone: (240) 276-6328
E-mail: urdanetaa@mail.nih.gov

5 P01 CA128814 07/22/09–06/30/14
NIH/NCI

PI Project 1: Ronai, Z. 1.2 calendar (10%)

PI Core A: Ronai, Z. 1.2 calendar (10%)

Targeting Pten - An Upstream, Downstream and Offstream Approach

Goals: To study the role of Pten and related downstream signaling components in melanoma development and progression.

Specific Aims: (Program 1) Determine and characterize mechanisms underlying the regulation and function of the ubiquitin ligase Siah in melanoma tumorigenesis and metastasis. (Program 2) Define aspects of central carbon metabolism that are regulated by Pten/Akt and Siah, and assess whether these metabolic hubs are valid drug targets in Pten null melanoma tumors. (Program 3) Use structure-based drug design to develop, characterize and validate novel small molecule antagonists against Akt and Siah2.

Grants Officer: Angela Urdaneta
National Institutes of Health
9000 Rockville Pike
Bethesda, MD 20892
Phone: (240) 276-6328

5 P30 CA30199

05/01/97–04/30/15

NIH/NCI

Associate Director: Ronai, Z.

1.2 calendar (10%)

Program Leader: Ronai, Z.

1.2 calendar (10%)

Cancer Center Support Grant

Goals: To provide director to the overall research mission pertaining to cancer research and to support shared service facilities, unique resources and recruitment of new staff members for the Institute's programs. This is an institutional grant, no funds from this grant are used to directly support Dr. Ronai's laboratory.

Grants Officer:

Latosha Mathis
National Cancer Institute
6120 Executive Blvd.
Rockville, MD 20852
Phone: (301) 496-3177
E-mail: latosha.mathis@nih.gov

Melanoma Research Alliance (PI: Ronai, Z.)

05/01/11–04/30/14

0.12 calendar (1%)

Combined Inhibition of NF-kappaB and AKT for Melanoma Treatment

Goals: This proposal focuses on the characterization of chemical compounds capable of inhibiting both AKT and NF-kappaB signaling pathways.

Specific Aims: (1) Validate effectiveness of BI-69-A11 in panel of 18 human melanoma cell lines, alone and in combination with B-Raf and MEK inhibitors. (2) Assess the effect of BI-69-A11 and B-Raf inhibitor on growth of human melanoma tumors transplants in mice. (3) Assess the effect of BI-69-A11 alone and with B-Raf inhibitor on development and metastasis of genetic mouse melanoma models.

Scientific Officer:

Laura Brockway-Lunardi
Melanoma Research Alliance
1101 New York Avenue, Suite 620
Washington, DC 20005
Phone: (202) 336-8937
E-mail: lbl@curemelanoma.org

Pending grants that are now active:

1 R01 CA188372-01A1 (PI: Ronai, Z.)

04/01/15–03/31/20

1.2 calendar (10%)

NIH/NCI

Understanding and Targeting the Glutamine Carrier SLC1A5 in Breast Cancer

Goals: The major goal is to advance the understanding of Gln metabolism in BCa and provide the foundation for novel stratification methods and therapeutic modalities for BCa.

Specific Aims: (1) Identify RNF5-dependent and -independent transcriptional, translational, and post-translational events regulating SLC1A5/38A2 availability and activity in representative BCa cultures. (2) Establish the biological significance of SLC1A5/38A2 expression in BCa cells for cellular metabolism, mitochondrial dynamics and function, autophagy, growth, and response to therapy. (3) Using BCa tumor samples, circulating tumor cells and TMAs we will determine the relationship between BCa expression of SLC1A5/38A2 and RNF5, the response to treatment, and disease outcome.

Scientific Review Officer:

Charles S. Morrow, M.D., Ph.D.
Tumor Cell Biology Study Section (TCB)
Oncology 1-Basic Translational (OBT)
NIH/Center for Scientific Review, Room 4192

6701 Rockledge Drive
Bethesda, MD 20892
Phone: (301) 408-9850
E-mail: morrowcs@csr.nih.gov

Martin Gleave, Partnering PI (Vancouver Prostate Centre)

Grants that have ended:

CIHR 244333 (PI: Burt, H) 7/1/11–6/30/14 0.36 calendar (3%)
Intravesicular Nanoparticulate Drug Delivery Systems for Application in Bladder Cancer

CIHR Role: Co-Investigator

Goals: The major goal of this project is about new information on mechanisms and processes of drug loaded amine-conjugated nanoparticulate induced urothelial exfoliation and repair, tumor cellular and tissue distribution and cellular effects critical to future design and success of intravesical therapies.

Specific Aims: (1) To develop suitable bladder tissue adhesive nanoparticulate delivery systems for anticancer agents. (2) To determine the factors influencing the interactions of untreated and surface modified nano-delivery systems with bladder tissue. (3) To evaluate efficacy of selected formulations administered into the bladders in animal models.

Grant's Officer: Benoit Lauzon
Grants and Awards Financial Officer Canadian Institutes of Health Research
160 Elgin Street
9th Floor Address Locator 4809A
Ottawa, ON, K1A 0W9
Telephone: 613-954-1949
Fax: 613-954-1800
Email: benoit.lauzon@cihr-irsc.gc.ca

Grant# n/a (PI: Cox, Gleave & Pandey, M) 7/1/12–6/30/14 0.36 calendar (3%)
Regulation of DNA Methyltransferases (DNMTs) by Gli Proteins and Its Effects on Progression of Prostate Cancer

Prostate Cancer Canada Movember Pilot Grant Role: Co-Investigator

Goal: the research proposed here will provide a novel insight into previously unexplored cross-talking that occurs between hedgehog-signalling and DNA methyltransferases in prostate cancer to facilitate tumour growth and survival.

Specific aims: (1) Correlate expression pattern of components of the hedgehog signaling pathway, DNMTs and CpG methylation loci profiles in different stages of prostate. (2) Characterize mechanism(s) of Gli regulation of DNMTs. (3) Identify genes regulated by Gli-induced activation of DNMTs in PCa.

Grant's Officer: Prostate Cancer Canada
Stuart Edmonds 2 Lombard Street
3rd Floor
Toronto Ontario M5C 1M1Canada
info@prostatecancer.ca
Telephone: 416-441-2131 Toll-free: 1-888-255-0333
Fax: 416-441-2325

Pending grants that are now active:

Movember 2015 Challenge Award 7/15-7/17
Prostate Cancer Foundation

Targeting Aberrant AR-FL and AR-V Expression and Activity to Overcome Therapy Resistance in Metastatic Castration-Resistant Prostate Cancer

Goals: The overall hypothesis being tested in this proposal is that aberrant PSA eRNA expression acts as a new proxy of AR functional abnormality that can be exploited for therapy of CRPC.

Specific aims: (1) To test this original hypothesis, we will test the hypothesis that aberrant upregulation of PSA eRNA and expression of its trans targets in CRPC metastases associate with disease progression during treatment with abiraterone acetate. (2) To determine the mechanism of action of PSA eRNA in supporting gene transcription activation and enzalutamide-resistant growth CRPC cells using TALEN-based gene editing. (3) To determine whether inhibition of PSA locus activity and AR expression overcomes enzalutamide-resistant growth of CRPC cells using preclinical mouse models. Success with the proposed aims will develop strong pre-clinical rationale for targeting PSA eRNA as a novel approach to inhibit AR transcriptional activity in CRPC.

Neil Bhowmick, Partnering PI (Cedars-Sinai Medical Center)

Grants that have ended:

n/a Chung (PI) 08/01/11–10/31/13 0.12 calendar (1%)

Prostate Cancer Foundation

Targeting cell death programs in the tumor and its microenvironment

Goals: The goals are to establish new molecular insights into the paracrine interaction between bone and prostate stromal cells in PCa metastatic progression and bone colonization, and determine an optimal therapeutic combination targeting the cell signaling network with XL-184 to attenuate the activation of the RANK and c-MET signaling axes, and to validate signaling mechanisms as potential biomarkers for the efficacy of prostate cancer therapy.

Specific aims: (1) Establish new molecular insights of the paracrine interaction between bone and prostate stromal cells for PCa metastatic progression and bone colonization. (2) Determine an optimal combination of therapeutic targeting of cell signaling network with XL-184 to attenuate the activation of RANK and c-MET signaling axes and their downstream signaling network converged at Src, PI3K-Akt, NFkB and Mcl-1. (3) Validate signaling mechanisms as potential biomarkers for the efficacy of prostate cancer therapy.

Role: Co-Investigator

Contracting/Grants Officer: Howard R. Soule, PhD
Executive Vice President, Chief Science Officer
Prostate Cancer Foundation
1250 Fourth Street
Santa Monica, CA 90401
Phone: 310-570-4596

n/a Isaacs/Karp/Bhowmick (PI) 10/15/12–10/14/14 0.12 calendar (1%)

Prostate Cancer Foundation

First-in-Man Clinical Studies of Mesenchymal Stem Cell Based Therapy for Prostate Cancer

Goals: Goals are to develop a mesenchymal stem cell delivery method for prostate cancer tested in mouse models and validated in neoadjuvant clinical trials in men.

Specific aims: (1) Pre-prostatectomy Phase 0 study to document homing of hbMSCs to sites of prostate cancer. (2) Pre-Clinical studies to determine optimal drug for delivery via microparticle loading hbMSC.

Contracting/Grants Officer: Howard R. Soule, PhD
Executive Vice President, Chief Science Officer
Prostate Cancer Foundation
1250 Fourth Street

Santa Monica, CA 90401
Phone: 310-570-4596

n/a Knudsen/Freeman (PI) 10/01/12–09/30/15 0.12 calendar (1%)
Spielberg Family Cancer Foundation
The Ecosystem of Lethal Prostate Cancer

The study involves the analysis of DNA methylation status of prostate cancer patient stromal cells in order to better characterize the cancer associated changes leading to lethal prostate cancer progression.

Aim 1: To identify genomic and transcriptomic signatures of adverse outcome of Gleason grade 4 (GG4) prostate cancer.

Aim 2: To test whether modified gold nano-rods can be used to quantify methionine metabolites in serum as a means of reporting adverse outcome in prostate cancer.

Aim 3: To test whether genomic and palmitoyl-proteomic profiles of a primary tumor can be measured in affinity-captured microvesicles.

Aim 4: To identify a signature of adverse outcome in circulating tumor cells (CTCs).

Role: Co-Investigator

R13DK101308 Bhowmick (PI) 09/15/13–01/31/14 0.3 calendar (2.5%)
NIH/NIDDK

Society for Basic Urologic Research (SBUR) 2013 Fall Symposium

Goals: The goal is to fund travel awards for selected trainees and junior investigators at SBUR Fall Symposium. The conference is held with goals of sharing new findings at a multidisciplinary level, promoting interaction among members and other interested scientists, inspiring and promoting the success of trainee scientists, and highlighting new areas of research and funding opportunities.

Contracting/Grants Officer: Ziya Kirkali, M.D.
NIDDK, National Institutes of Health
Building 2DEM, Room 627
6707 Democracy Blvd.
Bethesda, MD 20817
Phone: 301-594-7718

PC110699 Tripathi (PI) 09/30/12–09/29/14 0.0 calendar (0%)
DOD USMRMC Prostate Cancer Research Program

TGF β Mediated Regulation of HGF / c-met Signaling in the Progression of Castrate Resistant Prostate Cancer Bone Metastasis.

Goals: The goal is to identify the role of prostatic stromal fibroblastic cells in the support and differentiation of cancer stem cells in transgenic mouse models, xenografts, and human tissues.

Specific aims: (1) Elucidate the role of TGF β mediated HGF/c-Met stromal signaling in the induction or maintenance of stem like characteristics in PCa cells. (2) Elucidate the role of TGF β in mediating the HGF / c-Met signaling axis on the growth of PCa cells in the bone microenvironment and subsequent bone remodeling.

Role: Mentor

Contracting/Grants Officer: Kathy Robinson
USA Congressionally Directed Medical Research Program
Fort Detrick, MD
Phone: 301-619-8803

K01CA140711 Martinez-Ferrer (PI) 10/01/09–09/30/14 0.0 calendar (0%)
NCI Mentored Career Development Award
Inflammatory mediators of prostate cancer metastasis and responses to curcumin

Goals: This proposal tests the hypothesis that chemokine biomarkers that predict biochemical recurrence of prostate cancer regulate metastatic progression of the cancer and curcumin can inhibit metastasis of prostate cancer by antagonizing inflammatory signaling.

Pending grants that are now active:

2 P01 CA098912 (Chung/Bhowmick) 09/24/09–02/29/2020 1.2 calendar (10%)

National Institutes of Health

Prostate Cancer Bone Metastasis Biology and Targeting

Goals: This program project grant focuses on the elucidation of the biology and molecular pathways involved in the interaction between stromal cells of the bone or the prostate and malignant prostate cancer cells.

Specific aims for Project 3: (1) Define the role of CAFs in inducing and/or supporting MO-mimicry. (2) Evaluate interactions of MO-mimicry PC epithelia at the vascular and endosteal niche on bone engraftment. (3) Determine if detection of the MO-mimicry profile in diagnostic prostate biopsies is an indicator of clinical bone metastases and/or occult bone metastasis.

NCI Program Officer: Suresh Mohla, Ph.D.
Chief, Tumor Biology and Metastasis Research
National Cancer Institute
6130 Executive Blvd., Suite 5038
Bethesda, MD 20892-7364
mohlas@mail.nih.gov

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

This grant is a joint proposal with the following log numbers and respective award numbers. As such, we will be submitting duplicative reports.

CDMRP Log Number	Grant Agreement Number	Recipient	Principal Investigator
	W81XWH-14-1-0551	Sanford-Burnham Medical Research Institute	Ze'ev Ronai
PC130699P1	W81XWH-14-1-0552	Cedars-Sinai Medical Center	Neil Bhowmick
PC130699P2	W81XWH-14-1-0553	University of British Columbia	Martin Gleave

9. APPENDIX

Figures documenting the results obtained with Siah1/2 inhibitory peptides and results supporting the identification of small molecule inhibitors of Siah1/2 based on the PTS screen are provided in Figures 1–5 in Appendix 1. Figures depicting the studies in Dr. Gleave lab are shown in Figures 6–9. Data from Dr. Bhowmick lab is shown in Figures 10–12.

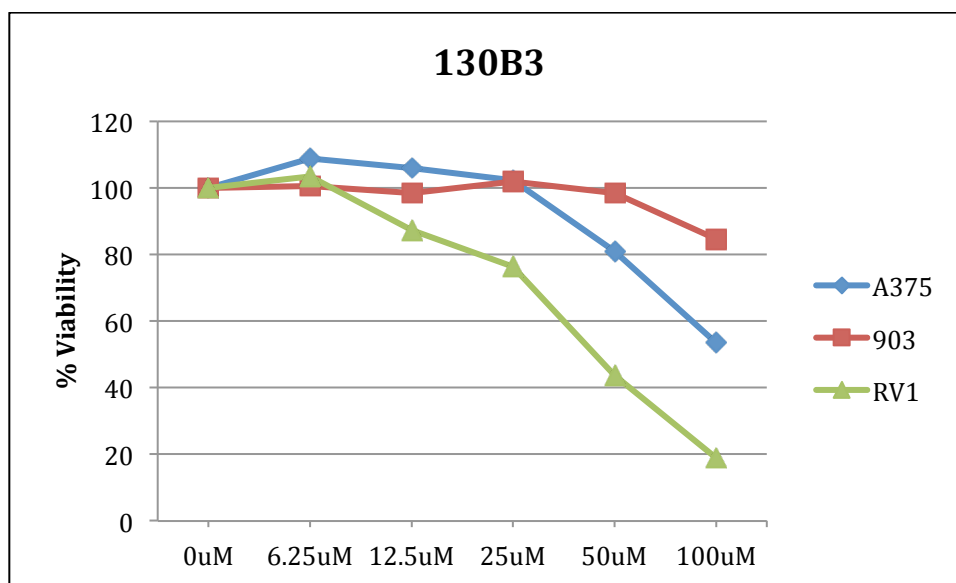


Figure 1. Inhibition of tumor growth by Siah2 inhibitory peptide 130B3. Peptide, at the indicated concentrations, was added to the melanoma (A375 and UACC903) and prostate cancer cell line CDW22RV1 (RV1). The most effective inhibition was seen on prostate cancer cells, within 48–72 h of treatment.

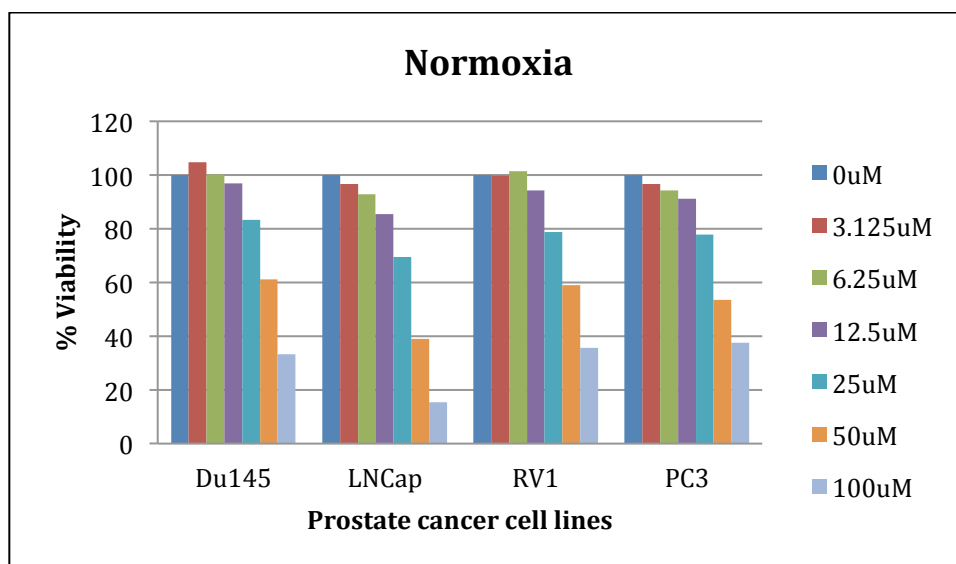


Figure 2. Dose dependent inhibition of prostate cancer cells following treatment Siah2 inhibitory peptide 130B3. Peptide, at the indicated concentrations was added to the four different prostate cancer cell lines and viability was assessed within 48–72 h after treatment.

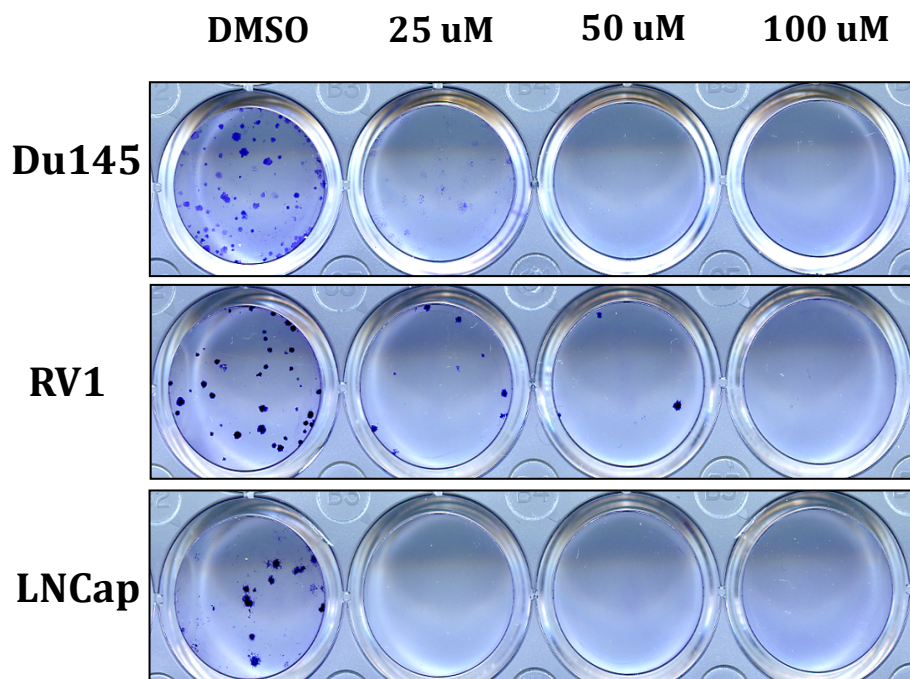


Figure 3. Dose dependent inhibition of prostate cancer cells ability to form colonies following treatment Siah2 inhibitory peptide 130B3. Peptide, at the indicated concentrations was added to 3 different prostate cancer cell lines and CFE was assessed within 5–7 days.

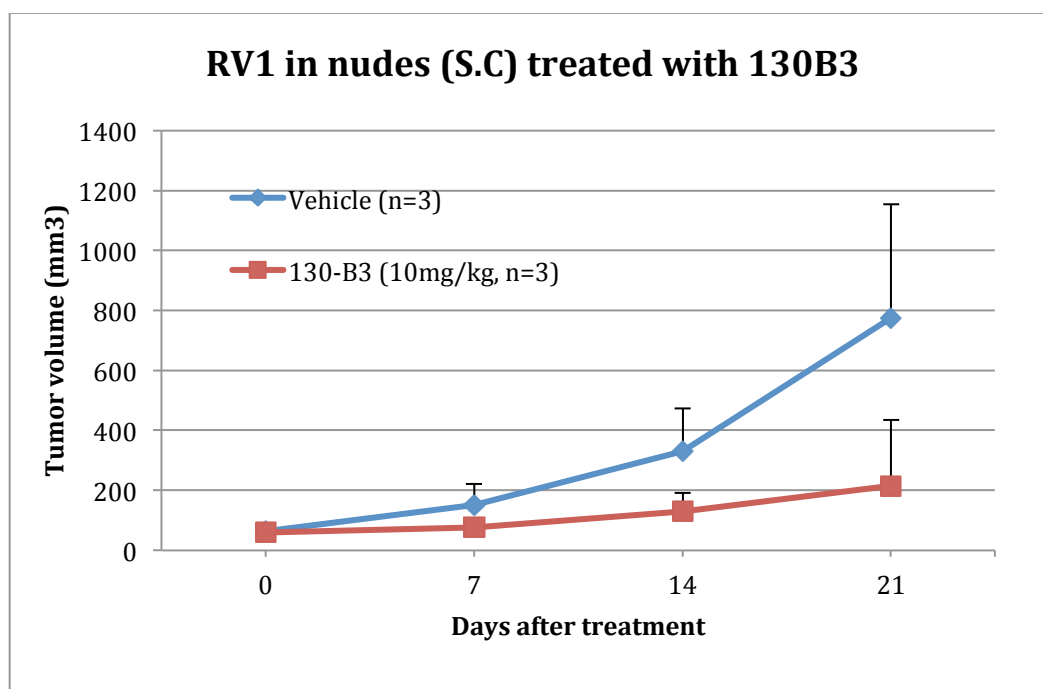


Figure 4. *In vivo* administration of 130B3 by iv effectively inhibits the growth of CDW22RV1 human adenocarcinoma of the prostate (administered by sc). Tumors were formed (a week after the injection) and were subjected to iv administration of 130B3 on daily basis for 5 days, with two days gap before additional 5 day treatment took place.

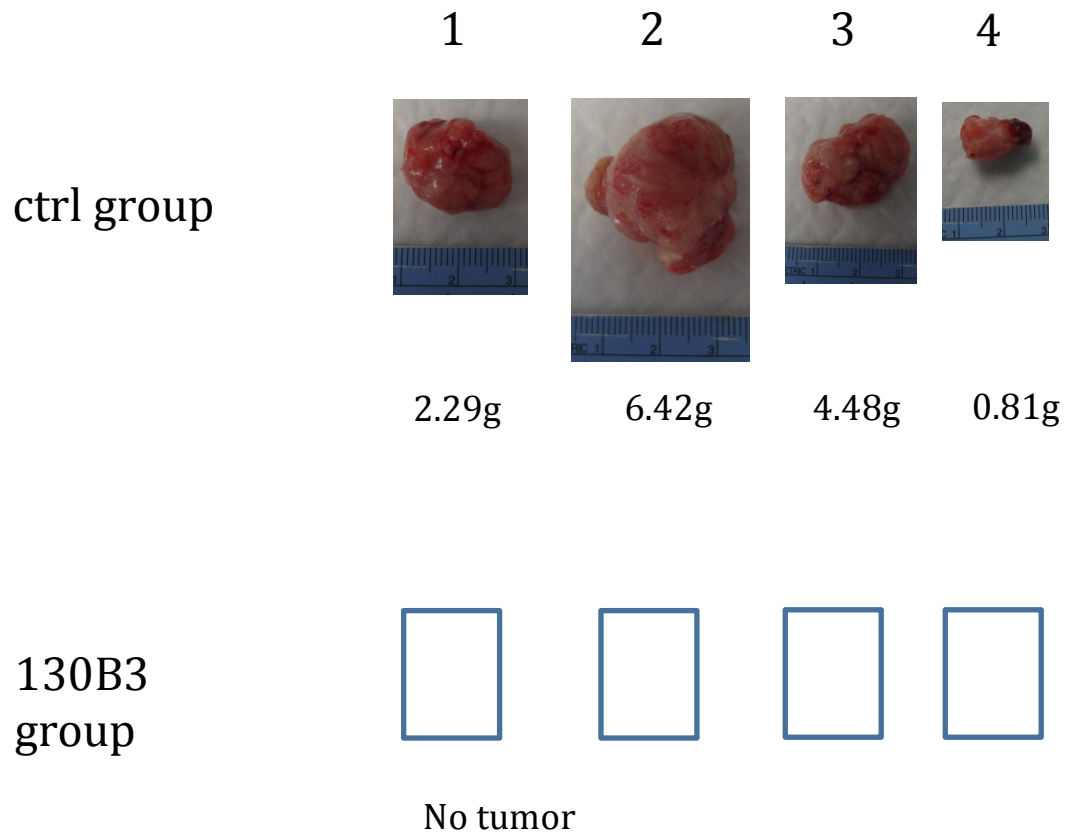
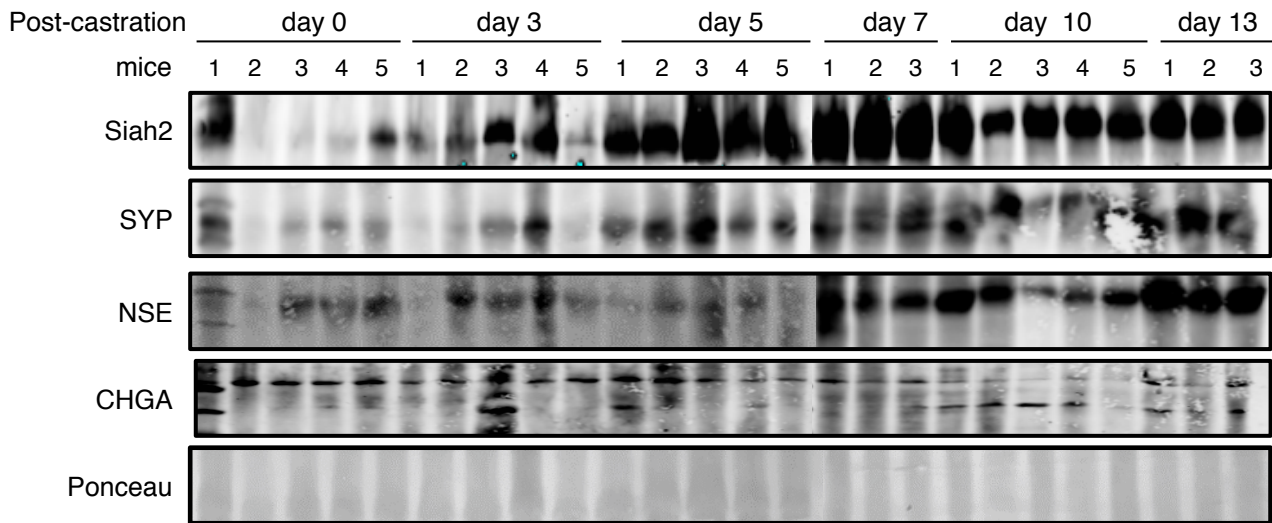
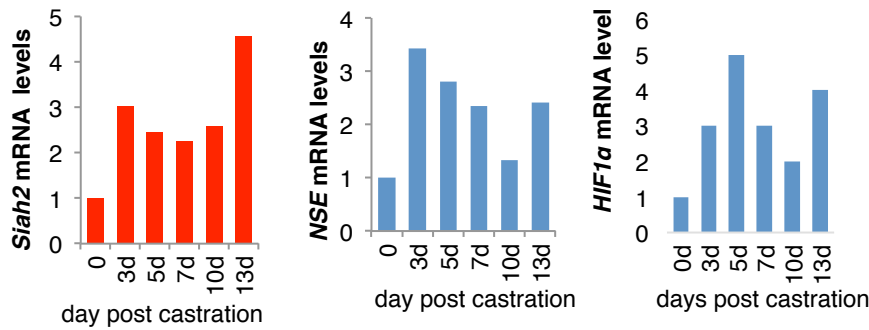


Figure 5. Administration of 130B3 inhibits the development of RV1, which were injected into the orthotopical prostate. One million RV1 cells were subjected to orthotopic prostate inoculation, and 10 days later animals were subjected to treatment with 130B3 that was administered by iv injections for 5 days, with 2 days break before additional administration was provided for the next 5 days. Tumor was harvested after one month, and corresponding tissue that lack of tumor was identified in the treated 130B3 group.

A



B



C

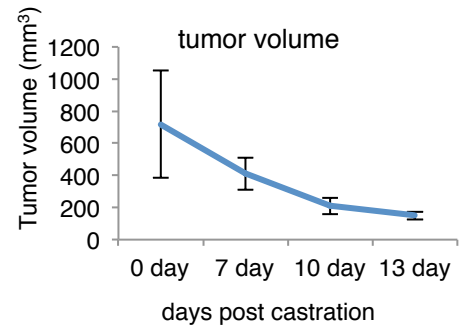
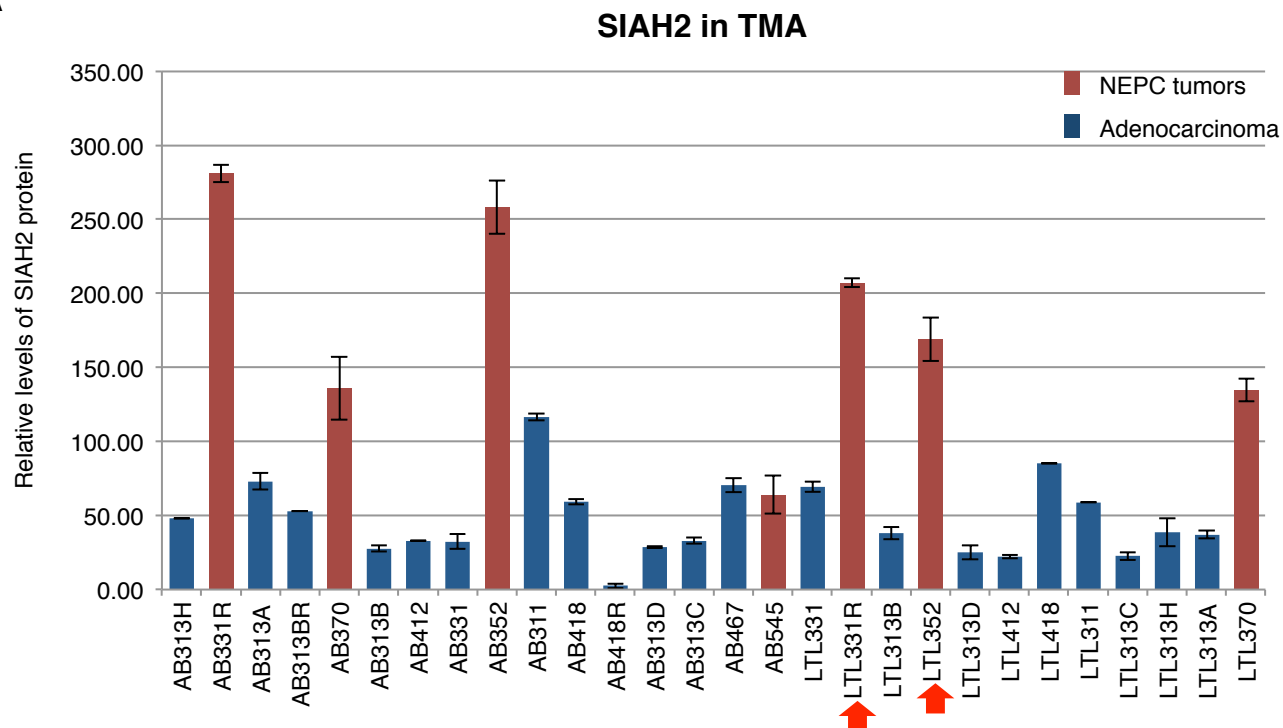


Figure 6. Castration induces mRNA and protein levels of Siah2 along with other markers for NEPC or ER stress in tumor tissues of Shionogi animal model. (A) Shionogi tumors were grown to palpable size in DD/S mice and then mice were castrated. Tumor tissues were harvested before castration (day 0) and post castration periods from Day 3 to day 13. Whole protein lysates were prepared from tumors and western blots were performed to investigate levels of the proteins as indicated. Ponceau S staining was used as loading control. (B) mRNA levels of Siah2, NSE and HIF1α were analysed with q-PCR in tumor samples collected from Shionogi model before and post castration. (C) Volume of tumors before and post castration.

A



B

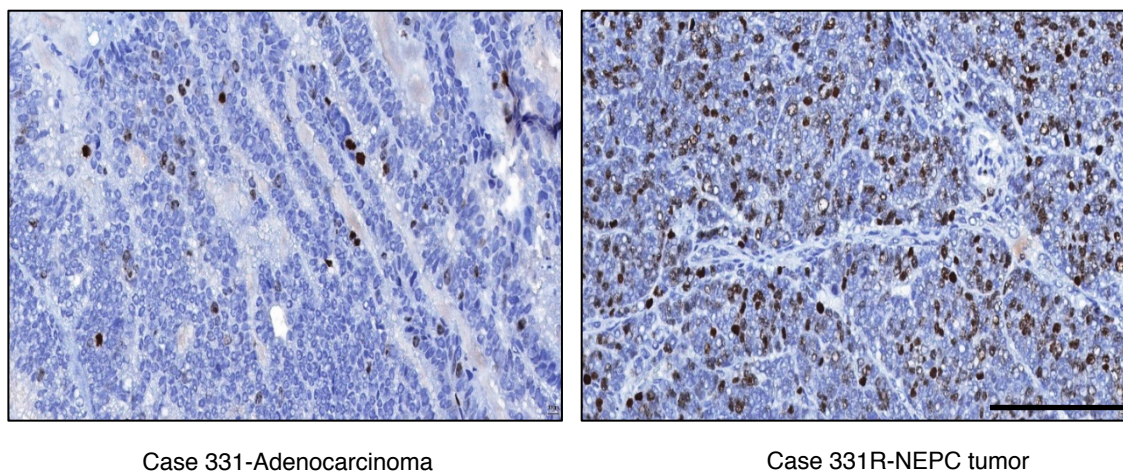
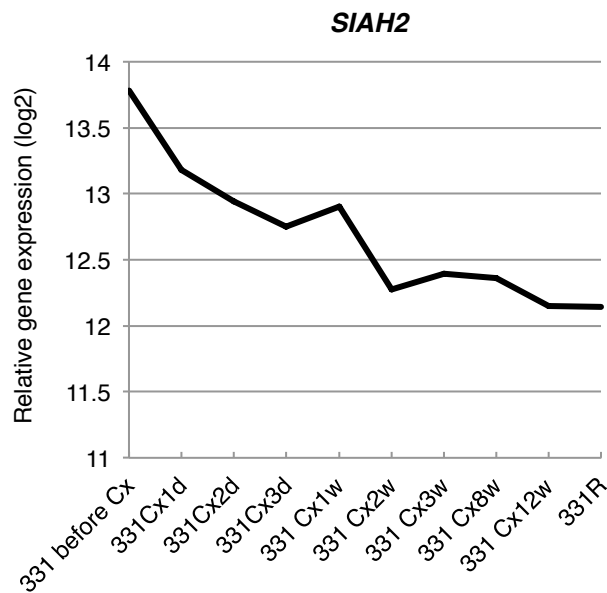


Figure 7. Siah2 protein levels are enhanced in PDX tumors detected by IHC. (A) Siah2 protein levels were examined with IHC staining in tissue micro array sets from patients' prostate adenocarcinoma or NEPC (set of 'AB') and PDX xenograft tumors (set of 'LTL'). Six out of 7 NEPC samples displayed enhanced Siah2 protein levels. Red arrows indicate two PDX xenografts LTL331R and LTL352 that will be recruited for the Siah2 *in vivo* inhibition assay. (B) Representative images of IHC staining for Siah2 protein in the TMA were shown. Scale bar: 100 μ m.

A

SIAH2 mRNA Levels Are Reduced
in PDX LTL331 castration series



B

SIAH2 mRNA Levels Are Not Changed in
Clinical NEPC Samples

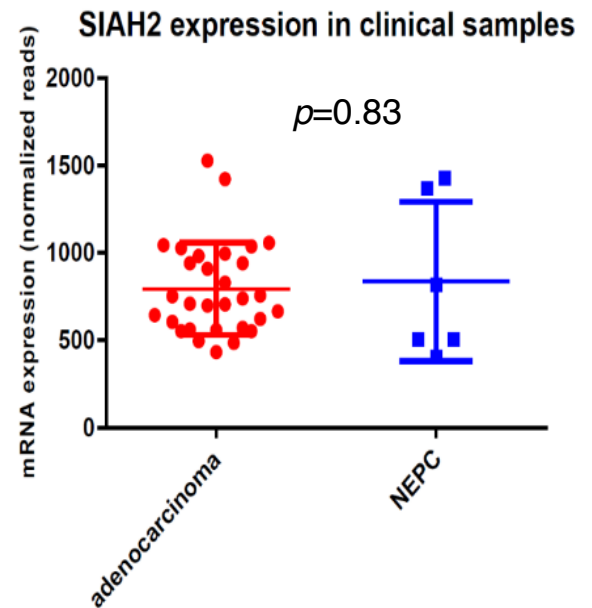


Figure 8. Siah2 mRNA levels are not enhanced in PDX xenograft and clinical samples. (A) Siah2 mRNA levels were analysed in PDX LTL331 model before castration (Cx) and post castration. Opposite with the observation on protein levels, the mRNA of Siah2 is reduced along with the castration. (B) Siah2 mRNA levels were screened in the Mark Rubin's clinical dataset. No significant difference was detected between adenocarcinoma and NEPC

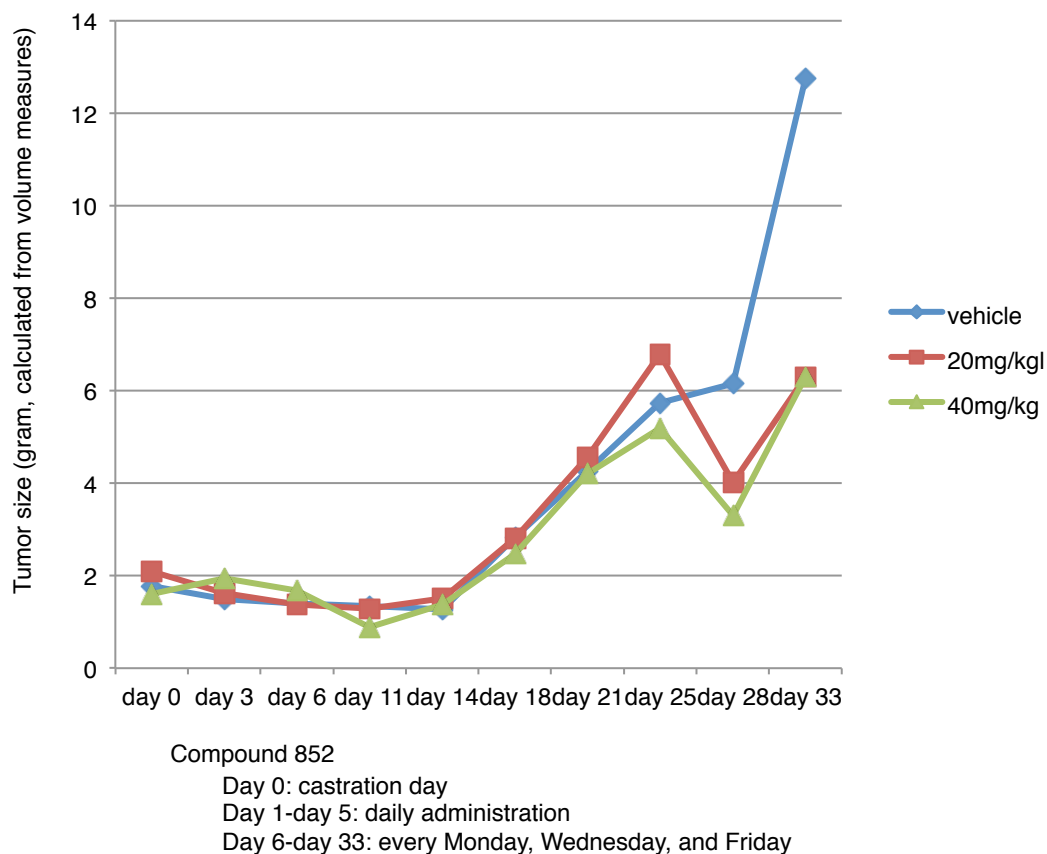


Figure 9. Effects of Siah2 inhibitor compound 852 on Shionogi tumor progression after castration. 5×10^6 of TD-2 cells were injected subcutaneously into DD/S mice. When tumors reach 500 mm^3 (2 to 3 weeks after injection) mice are castrated under anesthesia and randomly enter groups for vehicle, 20 mg/kg of compound 852 and 40 mg/kg of 852. Treatments were started the day after castration with i.p. injection, daily administration for the first 5 days and then three doses/week for the rest period. Tumor growth was continuously measured biweekly until the sacrifice date.

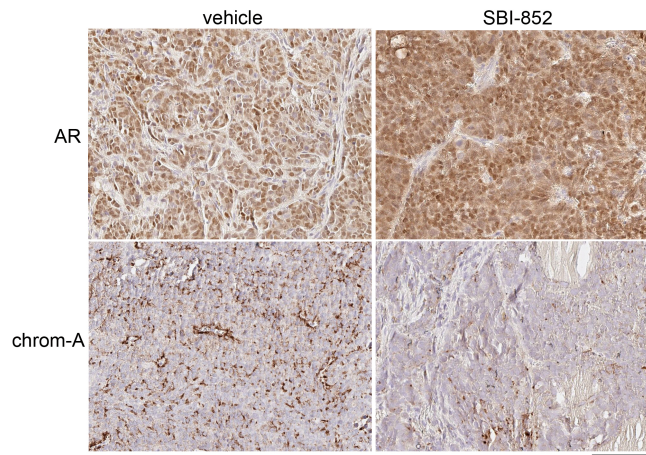


Figure 10. Immunohistochemistry for the expression of AR and chromogranin A (chrom-A) of PCa tumors from castrated mice treated with SBI-852. Scale bar indicates 100 μ m.

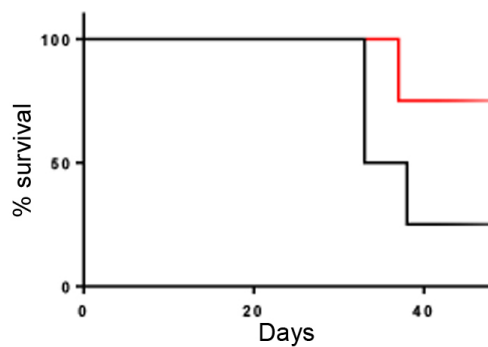


Figure 11. The survival of PCa tumor bearing mice treated with enzalutamide alone (black) vs. enzalutamide and 130B3 (red).

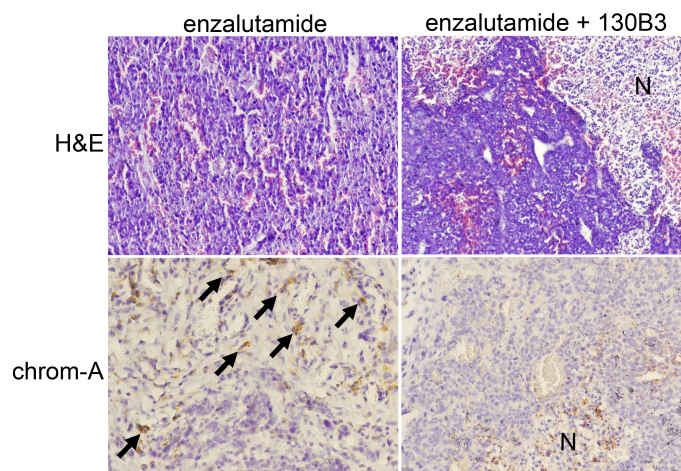


Figure 12. Histology of PCa tumors in mice treated with enzalutamide and enzalutamide + 130B3. Areas of necrosis are indicated with "N". Chromogranin A (chrom-A) immunolocalization is indicated by arrows.